



Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at <http://about.jstor.org/participate-jstor/individuals/early-journal-content>.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE PROTEIN POISON OF THE TONSIL *

W. H. BURMEISTER

(From the Department of Pathology, University of Illinois, Chicago.)

In 1913, Dick and I found that the extracts of tonsils were, in many instances, acutely toxic for animals when injected intravenously.¹ This toxic action manifested itself clinically like the reaction obtained in anaphylaxis. Bacteriological examinations showed that typical hemolytic streptococcus colonies could be obtained in the flora of the highly toxic tonsils whereas this organism was present in only 10 percent of the relatively non-toxic tonsils. It was noted, among other things, that, unlike the anaphylatoxin of Friedberger, the toxicity of these extracts was not destroyed by heating to 65 C., but that it was diminished without rendering them entirely non-toxic. A further consideration of this point has led me to believe that the toxicity of these extracts is dependent upon more than one factor. That the toxicity of these extracts is dependent, in part, upon an anaphylatoxin of bacterial extraction is not improbable. The nature of the heat resistant portion, however, is open to question.

With this in view a further study of tonsil extracts was undertaken. The tonsils were obtained through the courtesies of Dr. A. A. Hayden and Dr. Lillian E. Taylor. From a study of Table 1, it will again be seen that, in a large majority of the extracts of greatest toxicity, the streptococcus hemolyticus was present, and that it was usually absent or, at most, not the prevailing organism, in the tonsils from which the less toxic extracts were obtained.

From a gross examination of the tonsils, it was noted that tonsils with well-defined crypts, and especially those containing caseous plugs, were almost uniformly severely toxic. It was also observed, in most instances, that these extracts contained a greater amount of heat-non-coagulable-biuret reacting substances than did the less toxic tonsils. Without exception, all organ and tissue extracts contain such substances, but the marked increase in the depth of the biuret reaction was most striking when compared with that of the less toxic extracts.

* Received for publication September 1, 1914.
1. Jour. Infect. Dis., 1913; 13, p. 273.

TABLE 1
APPEARANCE AND FLORA OF TONSILS, AND ACTION OF EXTRACT

Tonsil	Gross Appearance of Tonsil	Bacterial Flora of Tonsil	Biuret Reaction of Tonsil Extract	Amount Injected	Action on Rabbit
1	Large, hyperplastic, no caseous plugs	Staphylococcus, micrococcus tetragenus, influenza bacillus	+	5 c.c.	Sneezed, urinated, rapid respiration, quiet, lived
2	Large, hyperplastic, crypts, caseous plugs	Hemolytic streptococcus, small punctate colonies, staphylococcus, streptococcus mucosus	++++	4 c.c.	Died 2 minutes
3	Large, hyperplastic, no caseous plugs	Staphylococcus, pneumococcus, hemolytic streptococcus, small punctate colonies, very few	+	6 c.c.	Sneezed, urinated, died during night
4	Large, hyperplastic, no plugs	Staphylococcus, streptococcus viridans, B. Friedlander, hemolytic streptococcus, small punctate colonies	+	10 c.c.	Lived, restless, rapid breathing
5	Large, hyperplastic, no plugs	Staphylococcus, streptococcus viridans, hemolytic streptococcus, small punctate colonies	++	9 c.c.	Lived, restless, rapid breathing, defecated, urinated
6	Small, fibrous, no plugs	Hemolytic streptococcus, small punctate colonies, streptococcus viridans, staphylococcus, pneumococcus	+++	5 c.c.	Died, 1 minute
7	Large, hyperplastic, scars, no plugs	Staphylococcus, hemolytic streptococcus, small punctate colonies	++++	5 c.c.	Died, 30 seconds
8	Large, hyperplastic, no plugs	Streptococcus viridans, staphylococcus, pseudodiphtheria bacilli, pneumococcus	++	10 c.c.	Lived, slight twitching
9	Large, hyperplastic, caseous plugs, scars	Hemolytic streptococcus, small punctate colonies, streptococcus viridans, staphylococcus, pneumococcus	++++	5 c.c.	Died, 30 seconds
10	Large, hyperplastic, no plugs	Hemolytic streptococcus, large, low, flat, wrinkled colony, staphylococcus	++	9 c.c.	Lived, slight twitching, scratched nose and ears
11	Large hyperplastic, caseous plugs	Hemolytic streptococcus, small punctate colonies, staphylococcus, pseudodiphtheria bacilli	++++	4 c.c.	Died, 1 minute
12	Large, hyperplastic, caseous plugs	Staphylococcus, hemolytic streptococcus, small punctate colonies	++	10 c.c.	Died, 8 minutes
13	Small, fibrous	Staphylococcus, streptococcus viridans, micrococcus catarrhalis	+	5 c.c.	Lived, quiet, urinated, defecated
14	Large, hyperplastic, caseous plugs	Hemolytic streptococcus, large, low, flat, wrinkled colony, hemolytic streptococcus, small, punctate colonies, staphylococcus alba	++++	5 c.c.	Died, 30 seconds
15	Small, fibrous, scars	Staphylococcus, pneumococcus, hemolytic streptococcus, small punctate colonies	++	5 c.c.	Died, 3 minutes
16	Large, hyperplastic, no plugs	Hemolytic streptococcus, small punctate colonies, staphylococcus, pneumococcus	+++	5 c.c.	Died, 7 minutes
17	Small, fibrous, no crypts	Influenza bacillus, micrococcus tetragenus, pneumococcus, staphylococcus	++++	5 c.c.	Died, 1 minute
18	Large, hyperplastic, crypts, caseous plugs	Hemolytic streptococcus, large, low, flat, wrinkled colony, hemolytic streptococcus, small punctate colonies, staphylococcus alba	+++++	5 c.c.	Died, 1 minute
19	Small, hyperplastic, crypts, caseous plugs	Pneumococcus, non-hemolytic streptococcus, small punctate colonies, staphylococcus	+++	5 c.c.	Died, 2 minutes

TABLE 1—Continued

20	Large, hyperplastic, crypts, caseous plugs	Pneumococcus, hemolytic streptococcus, small punctate colonies, streptococcus viridans, staphylococcus	+++++	5 c.c.	Died, 1 minute
21	Large, fibrous, no plugs	Staphylococcus, pneumococcus, hemolytic streptococcus, large, low, flat, wrinkled colony	++	5 c.c.	Lived, rubbed nose, face and ears, urinated, defecated, rapid respiration
22	Very large, hyperplastic, caseous plugs	Hemolytic streptococcus, large, low, flat, wrinkled colony, staphylococcus	+++++	5 c.c.	Died, 15 seconds
23	Large, hyperplastic, crypts, no plugs	Streptococcus viridans, staphylococcus, hemolytic streptococcus, small punctate colonies, few scattered	++	5 c.c.	Lived, scratched nose and ears, urinated, defecated, slight twitching
24	Large, hyperplastic, no caseous plugs	Hemolytic streptococcus, large, low, flat, wrinkled colony, staphylococcus aureus	+++	5 c.c.	Lived, rubbed nose, sneezed, urinated, panting respiration
25	Large, hyperplastic, caseous plugs	Hemolytic streptococcus, large, low, flat, wrinkled colony, B. Friedländer, staphylococcus	+++++	5 c.c.	Died, 30 seconds
26	Large, hyperplastic, no caseous plugs	Staphylococcus, streptococcus viridans	++++	5 c.c.	Lived, sneezed, rubbed ears, face and nose, urinated, defecated, panting respiration
27	Large, hyperplastic, caseous plugs	Pneumococcus, staphylococcus	+	5 c.c.	Lived, urinated, no other symptoms
28	Large, hyperplastic, crypts, no plugs	Staphylococcus, hemolytic streptococcus, large, low, flat, wrinkled colony, incomplete hemolysis	++	5 c.c.	Lived, panting respiration, no other symptoms
29	Large, hyperplastic, no caseous plugs	Staphylococcus, non-hemolytic streptococcus, small punctate colonies, pseudodiphtheria bacilli	+	5 c.c.	Lived, shallow rapid respiration, no other symptoms
30	Large, hyperplastic, caseous plugs	Streptococcus viridans, pneumococcus, staphylococcus	+++++	5 c.c.	Lived, shallow rapid respiration, no other symptoms
31	Large, hyperplastic, caseous plugs	Staphylococcus, streptococcus viridans	++	5 c.c.	Lived, sneezed, urinated, defecated, rubbed nose and face, shallow panting respiration
32	Small, fibrous, no caseous plugs	Staphylococcus, streptococcus viridans	+	5 c.c.	Lived, sneezed, defecated, panting respiration, rubbed nose
33	Medium sized, no plugs	Staphylococcus, pneumococcus, hemolytic streptococcus, small punctate colonies	+++	5 c.c.	Died, 3 minutes
34	Hyperplastic, caseous plugs	Hemolytic streptococcus, small punctate colonies, staphylococcus	+++	5 c.c.	Died, 1 minute
35	Very large, hyperplastic, no plugs	Streptococcus viridans, staphylococcus, hemolytic streptococcus, small punctate colonies	++	5 c.c.	Died, 1 minute
36	Large, hyperplastic, caseous plugs	Hemolytic streptococcus, small punctate colonies, staphylococcus	+++	5 c.c.	Died, 1 minute
37	Medium sized, no plugs	Staphylococcus, streptococcus viridans, pneumococcus	+	5 c.c.	Lived, sneezed, urinated, dyspnea
38	Medium sized, no plugs	Staphylococcus. B. Friedländer, non-hemolytic streptococcus, large, low, flat, wrinkled colony	++	5 c.c.	Lived, rubbed nose, sneezed, urinated, defecated, panting respiration
39	Small, fibrous, no plugs	Staphylococcus, micrococcus catarrhalis	+	5 c.c.	Lived, urinated, sneezed
40	Large, hyperplastic, caseous plugs	Staphylococcus, hemolytic streptococcus, small punctate colonies	+++++	5 c.c.	Died, 15 seconds

Those organisms which were present in greatest numbers are listed in the order of their frequency. The number of + signs express the relative degree of positive reaction of the individual extracts.

In determining this reaction the extracts were treated according to the method of Pfeiffer and Mita.² Less than 4 c.c. of the extract were diluted to ten times its volume with water and placed in an Erlenmeyer flask. This was immersed in boiling water and a very dilute acetic acid was added, drop by drop, until the mixture just reacted acid to litmus. It was found that before this point was reached the mixture invariably separated into coarse coagula and a water clear fluid, which could be filtered very readily. This was, by far, the more important consideration, and when the coagulation and separation were found to have taken place the addition of acid was stopped. The filtrate was then heated 15-30 minutes longer in the water bath, and tested with nitric acid for albumen. In all instances, the ring test was negative. Twenty cubic centimeters of the filtrate were then evaporated to 2 c.c. and tested with biuret reagent.

With few exceptions, the stronger biuret reactions were obtained with the more toxic extracts, altho biuret reactions of varying degrees and intensities were obtained in all extracts, as shown by Table 1. In other words, the more toxic extracts contained a larger amount of peptone-like products.

Biedl and Krauss,³ in trying to determine the nature of anaphylatoxin, found that, by injecting peptones into animals, a condition of shock was produced which resembled anaphylaxis. Vaughan and Wheeler⁴ were able to obtain, from the colon bacillus and other pathogenic and non-pathogenic bacteria, a protein-split product of marked toxicity which, when injected intravenously in doses of 0.5 mg., killed guinea-pigs under conditions resembling anaphylaxis clinically. They called this toxic substance "the protein poison" and advanced the theory that symptoms, due in general to bacterial disease, could be explained on the basis of a parenteral splitting of the bacterial proteins into toxic substances, akin to peptones. This theory has been accepted by many workers in the field of infectious diseases. Provided the individual has become sensitized, that is, has developed the specific lytic bodies for a certain protein, symptoms of protein poison can be produced in such an individual by the parenteral ingestion of the same protein. This is possible not only for bacterial proteins, but for proteins in general. It has been possible to sensitize animals and to produce the symptoms of protein poisoning with proteins derived from their own tissues. Schittenhelm and Stroebel⁵ found that the action of pepsin on species-similar tissue resulted in the formation of toxic-split products. Heyde and Vogt,⁶ by a series of very interesting experiments, have shown

2. Ztschr. f. Immunitätsf., 1910, 6, p. 18.

3. Wien. klin. Wchnschr., 1909, 10, p. 363.

4. Jour. Am. Med. Assn., 1913, 61, p. 1761.

5. Ztschr. f. exper. Path. u. Therap., 1912, 11, p. 108.

6. Ztschr. f. d. ges. exper. Med., 1913, 1, p. 59.

that death, in cases of burns, resulted from aseptic tissue splitting, and the production of a condition resembling anaphylactic shock as a result of the absorption of the toxic products. They also found that by crushing the animal's own tissue it was possible to sensitize the individual to this tissue.

By applying Abderhalden's dialyzing and optical methods for the detection of protein splitting by serum in different conditions, a great volume of experimental work has been produced, indicating that specific homologous proteolysis does occur and may be an important factor in the clinical manifestations of disease. Rollman⁷ advances the theory that disease of one of a pair of organs, especially in suppurations, results in the production of specific proteolytic ferments, which are capable of producing destructive changes in the other organ. It would seem not unlikely that the parenteral absorption of tissue proteins and their parenteral splitting, or the absorption of peptones, products of their splitting in loco, must be of some moment. This does not apply only to paired organs, but is equally applicable to a continued breaking down of tissue in any part of the body.

With this in mind, it was determined to ascertain the splitting power of the sera of individuals, the subjects of chronic and recurrent tonsillitis, on their own tonsil tissue, as well as on other tissue; and to determine, if possible, whether or not there is any proportional relationship between this splitting power, the toxicity of the tonsillar extracts, and their content of peptone-like bodies.

The patients were bled just previous to the tonsillectomy. The tonsils, after removal, were washed as free from blood as possible in a running stream of water. Each pair of tonsils was then rubbed up in a sterile mortar with 20 c.c. of physiological salt solution under aseptic conditions. The extracts were then decanted, vigorously centrifuged at 3,000 revolutions per minute for 10-15 minutes, and again decanted. Both residues were then boiled in water, according to Abderhalden's method of preparing placental tissue,⁸ until they were free from water-soluble ninhydrin and biuret reacting substances. About 1 gm. of the prepared tonsil tissue was then placed in a dialyzing sac, and covered with 1.5 c.c. of the serum. The sac and its contents were then placed in a receptacle containing 20 c.c. of distilled water; both water and contents of the sac were covered with a layer of toluene, and placed in an incubator at 37 C. for 16-18 hours. The dialyzing sacs were prepared from a thin 6-7 percent solution of celloidin in a mixture of equal parts of alcohol and ether. Only such celloidin was used as would permit a dialysis from a solution of silk peptone and would retain albumens, as evidenced by a failure of dialysis of biuret

7. Deutsch. med. Wchnschr., 1913, 39, p. 2239.

8. Handb. d. Biochem. Arbeitsmethoden, 1912, 6, p. 226.

TABLE 2
PROTEOLYTIC POWER OF SERUM AS COMPARED WITH ACTION TONSILLAR EXTRACT

Tonsil	Tonsillar Extract		Splitting Power of Active Serum on Homologous Tonsil	Splitting Power of Active Serum on Heterologous Tissue	Splitting Power of Rabbit Serum on Tonsil and Other Tissue
	Amount	Action on Rabbit			
1	5 c.c.	Slight, lived	B+ N+
2	4 c.c.	Killed	B+ N+ N+ N++
4	10 c.c.	Lived	B- N-
5	9 c.c.	Lived	B+ N+ N+ N++	Tonsil 7, B+, N++
6	5 c.c.	Killed	B+ N+ N++	Tonsil 7, B++++, N++++
7	5 c.c.	Killed	B+ N+	Tonsil 5, B-, N++
8	10 c.c.	Lived	B+ N+ N++	Tonsil 11, B++, N++++
10	9 c.c.	Lived	B- N+	Kidney B-, N++ Lymph-gland B-, N-
11	4 c.c.	Killed	B- N++	Lymph-gland B-, N+ Kidney B-, N++ Tonsil 8, B-, N+
16	5 c.c.	Killed	B- N-	Spleen B-, N- Thymus B-, N- Kidney B-, N- Ovary B-, N-	Tonsil 16 B-, N- Kidney B-, N-
17	5 c.c.	Killed	B+ N+ N++	Thymus B+, N++	Tonsil 17 B++++, N++++ Thymus B-, N-
18	5 c.c.	Killed	B- N+	Thymus B-, N-	Tonsil 18 B-, N-
19	5 c.c.	Killed	B+ N+ N+ N++	Thymus B+, N++ Spleen B-, N++ Kidney B++++, N++++ Ovary B-, N+	Tonsil 19 B-, N+ Thymus B-, N++
20	5 c.c.	Killed	B- N++	Thymus B++++, N++++ Spleen B-, N++ Kidney B-, N+	Tonsil 20 B-, N++ Spleen B-, N- Kidney B-, N-
21	5 c.c.	Lived	B- N-	Tonsil 21 B-, N-

TABLE 2—Continued

22	5 c.c.	Killed	B+++ N+++++	Tonsil 22 B—, N—
23	5 c.c.	Lived	B—	Tonsil 23 B—, N—
24	5 c.c.	Lived	B—	Tonsil 24 B—, N—
25	5 c.c.	Killed	B—	Tonsil 25 B—, N—
26	5 c.c.	Lived	B+++ N+++++	Tonsil 26 B—, N++
27	5 c.c.	Lived	B—	Kidney B—, N—	Tonsil 27 B—, N++
28	5 c.c.	Lived	B—	Kidney B—, N—	Tonsil 28 B—, N++
29	5 c.c.	Lived	B—	Kidney B—, N—	Tonsil 29 B—, N—
30	5 c.c.	Lived	B—	Kidney B—, N—	Tonsil 30 B—, N+
31	5 c.c.	Lived	B+++ N+++++	Kidney B—, N—
32	5 c.c.	Lived	B—	Liver B—, N++
33	5 c.c.	Killed	B— N+++++	Liver B—, N—
34	5 c.c.	Killed	B+++ N+++++	Liver B—, N++
35	5 c.c.	Killed	B—	Liver B—, N—
36	5 c.c.	Killed	B—	Liver B—, N—
37	5 c.c.	Lived	B— N+++++	Liver B—, N—
38	5 c.c.	Lived	B+++ N+++++	Liver B—, N—
39	5 c.c.	Lived	B—	Liver B—, N—
40	5 c.c.	Killed	B— N+++++	Liver B—, N—

+— = doubtful; — = negative.
 B = biuret reaction of dialysate; several + signs = relative degree of reaction.
 N = ninhydrin reaction of dialysate.

and ninhydrin reacting substances from normal blood serum.⁹ In compliance with the recommendation of Abderhalden¹⁰ not over 1.5 c.c. of serum were used in any of the experiments. The results have been recorded in Tables 2, 3, and 4.

From a study of Table 2 it will be seen that 12 of the 16 most toxic tonsils, the extracts of which killed acutely, were split by the serum obtained from the donors of the tonsils; and that, in all instances, these were tonsils the extracts of which had a large peptone content. From a comparison of Tables 1 and 2 it will also be seen that, in most instances, these were cases in which the tonsils presented well-marked crypts with caseous plugs. Table 2 also shows that, in the 18 less toxic tonsils, the serum-splitting action on tonsil tissue was absent in 9 cases and present in 9 cases; if further comparison of Tables 1 and 2 is made, it is seen that the extracts of the latter were probably of greater toxicity than those of the former, as evidenced by the degree of reaction obtained in the animal experiments, altho these extracts were not sufficiently toxic, in any instance, to produce death acutely with the amounts injected. It will be noted, however, that the content of heat-non-coagulable biuret-reacting substances was no greater in either of these two groups, demonstrating that the peptone content of these extracts cannot be the only factor upon which the toxicity of tonsils depends.

Suitable control experiments were made, and from the record of these in Table 2 it is seen that the more active sera split with equal ease tonsils other than those of the serum donor. It will also be noted that, in some instances, the control tests with other than tonsillar tissue presented positive, tho less marked reactions. Therefore, it appears that either this splitting action is not strictly specific, or else that more than one specific ferment may have been contained in the sera. Thus, it was found that controls of thymus tissue, spleen, ovary, lymph gland, and kidney were occasionally split by the same serum, tho in practically no instance to so marked a degree.

Another point which necessitated consideration was the possibility that the toxicity of these extracts was due to a parenteral splitting action of the proteins of the tonsil extracts by the rabbit serum after the injection into the rabbit. To test this possibility, the rabbits were bled by cardiac puncture to the extent of 6-7 c.c. of blood, on the day previous to their injection. They were given 15 c.c. of physiological salt solution, hypodermatically, following their bleeding, and furnished

9. *Ibid.*, p. 230.

10. *München. med. Wchnschr.*, 1914, 61, p. 401.

TABLE 3
ANALYSIS OF MECHANISM OF PROTEOLYTIC ACTION

Tonsil	Action of Active Serum on Homologous Tonsil	Action of Inactive Serum on Homologous Tonsil	Action of Inactive Serum Plus Complement on Homologous Tonsil	Action of Comple- ment on Same Tonsil and on Other Tissue	Action of Inactive Serum on Heterologous Tissue	Action of Inactive Serum Plus Comple- ment on Heterologous Tissue	Tonsillar Extract Injected	
							Amount	Action on Rabbit
21	B— N—	B— N—	B— N—	Tonsil B—, N— Lymph gland B—, N—	Lymph gland B—, N—	Lymph gland B—, N—	5 c.c.	Lived
22	B+++ N++++	B— N—	B+—? N++	Tonsil B—, N— Lymph gland B—, N—	Lymph gland B—, N—	Lymph gland B—, N—	5 c.c.	Died
23	B— N—	B— N—	B— N—	Tonsil B—, N— Lymph gland B—, N—	Lymph gland B—, N—	Lymph gland B—, N—	5 c.c.	Lived
24	B— N—	B— N—	B— N—	Tonsil B—, N— Lymph gland B—, N—	Lymph gland B—, N—	Lymph gland B—, N—	5 c.c.	Lived
25	B— N—	B— N—	B— N—	Tonsil B—, N— Lymph gland B—, N—	Lymph gland B—, N—	Lymph gland B—, N—	5 c.c.	Died
26	B+++ N++++	B— N—	B+ N+	Tonsil B—, N— Kidney B—, N—	Kidney B—, N—	Kidney B—, N—	5 c.c.	Lived
27	B— N—	B— N—	B— N—	Tonsil B—, N— Kidney B—, N—	Kidney B—, N—	Kidney B—, N—	5 c.c.	Lived
28	B— N—	B— N—	B— N—	Tonsil B—, N— Kidney B—, N—	Kidney B—, N—	Kidney B—, N—	5 c.c.	Lived
29	B— N—	B— N—	B— N—	Tonsil B—, N—	5 c.c.	Lived
30	B— N—	B— N—	B— N—	Tonsil B—, N— Kidney B—, N—	Kidney B—, N—	Kidney B—, N—	5 c.c.	Lived
31	B+++ N++++	B— N—	B— N+	Tonsil B—, N— Kidney B—, N—	Kidney B—, N—	Kidney B—, N—	5 c.c.	Lived
32	B— N++	B— N—	B— N++	Tonsil B—, N— Liver B—, N—	5 c.c.	Lived
33	B—+? N++++	B— N—	B+ N++	Tonsil B—, N— Liver B—, N—	5 c.c.	Died
34	B+++ N++++	B— N—	B+ N+++	Tonsil B—, N—	5 c.c.	Died
35	B— N—	B— N—	B— N—	Tonsil B—, N—	5 c.c.	Died
36	B— N—	B— N—	B— N—	Tonsil B—, N—	5 c.c.	Died
37	B— N++	B— N—	B— N+	Tonsil B—, N—	5 c.c.	Lived
38	B+ N++++	B— N—	B— N+	Tonsil B—, N—	5 c.c.	Lived
39	B— N—	B— N—	B— N—	Tonsil B—, N—	5 c.c.	Lived
40	B— N++	B— N—	B— N+	Tonsil B—, N—	5 c.c.	Died

B = biuret reaction of dialysate; +— = doubtful; — = negative, several + signs = relative degree of reaction.

with an abundance of food and water. They appeared perfectly normal on the following day. The serum, after being separated from its coagulum under aseptic precautions, was placed in a refrigerator until the following day and its splitting power determined for the tonsils, the extracts of which were injected. In 3 of the 7 rabbits, which were acutely killed, there was a splitting action in vitro of the tonsil tissue by the rabbit serum. In one of these three, where thymus tissue was used as a control, it was also found that the thymus tissue was split by the rabbit serum. I mention this because it was noted, in addition to the other results typical of anaphylaxis in rabbits, that in practically all acute deaths of rabbits in these experiments small hemorrhages were found in the thymus. The sera of 4 of 8 rabbits, which did not die, split in vitro the tonsil tissue with which they were subsequently injected. From Tables 1 and 2 it is seen that the symptoms manifested by these rabbits, whose sera had a splitting action, were, in a degree, somewhat more marked than those of the remaining four, whose sera failed to split the tonsil tissue.

That the tonsil substrates used must have contained also bacterial proteins of the tonsillar flora is of course true. It, therefore, seemed plausible that some of the evidences of protein-splitting in these substrates might, in part at least, have been due to the action of the serum on the contained bacterial proteins. With this in mind, control experiments of 9 cases were made as follows: 5 drops of tonsil extract, which had not been centrifuged, were smeared evenly over the surfaces of each of 3 blood-agar plates. These were incubated 18 hours and the surface growth washed off with salt solution. The bacteria were then prepared and used as a substrate for their respective tonsils. In these experiments, the amount of bacterial protein must have been many times greater than the amount that may have remained in the tonsil substrates. As seen in Table 4, in only two instances could any evidence of splitting be obtained and in no one instance did this tend to approach the amount of splitting obtained from the tonsil substrates.

It seemed advisable to determine further, if possible, the nature of this splitting action of serum from patients, the subjects of chronic tonsillitis on tonsil tissue. Dick,¹¹ in a study of the proteolytic substances in the blood, found that this proteolysis was almost entirely inhibited by heating the serum at 56 C. for one-half hour; and that it returned in part when complement was added to the heated serum.

11. Jour. Infect. Dis., 1911, 9, p. 282.

I have found that, when serum from patients is heated at 56 C. for one-half hour, the splitting-action on tonsillar tissue is completely inhibited. If, however, the serum is "activated" by the addition of guinea-pig complement, tonsillar tissue is again split, as will be seen from Table 3. Guinea-pig complement, alone, was, in no instance, capable of splitting tonsil tissue. Table 3 shows that the splitting power of the heated serum plus complement failed to equal in intensity the power of the active serum. In all cases in which the active serum failed to split tonsil tissue, the action of inactive serum plus complement, as was to be expected, was also negative.

TABLE 4
ACTION OF SERUM ON TONSIL AND ON TONSILLAR BACTERIA

Tonsil	Splitting Action of Serum on Homologous Tonsil	Splitting Action of Serum on Bacteria from Homologous Tonsil	Tonsil	Splitting Action of Serum on Homologous Tonsil	Splitting Action of Serum on Bacteria from Homologous Tonsil
32	B— N++	B— N—	37	E— N++	B— N—
33	B—+? N++++	B— N+	38	B+ N++++	B— N+
34	B++ N+++++	B— N—	39	B— N—	B— N—
35	B— N—	B— N—	40	B— N++	B— N—
36	B— N—	B— N—

In conclusion, then, it may be said that the hemolytic streptococcus is present in tonsils, the extracts of which are most toxic, and it is logical that the extracts' toxicity must depend, in part, on toxic products elaborated out of the streptococcus itself, or by the action of the streptococcus out of the tissue in which it is growing; or, that both hypotheses may be true. A further study of this phase should be undertaken.

The tonsils, the extracts of which are most toxic, contain a considerable amount of heat-non-coagulable biuret reacting substance or substances, in all probability a product of the splitting of proteins in loco. This splitting, no doubt, is due in part to the action of saprophytic or pathogenic micro-organisms on the tissue of the tonsil. It is not improbable, however, that part of this splitting action may be due also to the action of a ferment for tonsillar tissue contained in the blood, and which resembles an amboceptor and requires complement to

complete its action. It is plausible to assume that local death of tonsillar tissue by the action of organisms, like the streptococcus, permits of the parenteral absorption of tonsil protein, and results in the formation of an amboceptor for this protein which, with the aid of the complement, is capable of splitting the protein. This splitting action, then, might occur either in loco, or, following the parenteral absorption of tonsil protein, in the circulating blood. The toxic action of tonsillar products may then, in part at least, be responsible for many of the clinical symptoms manifested in the course of disease of the tonsils. The individual becoming sensitized to his tonsils exhibits, from time to time, mild or severe symptoms due to the toxic products of tonsillar origin.

The effect on the individual of continued sensitization in this manner can only be speculated on. Longcope has been able to produce an interstitial hepatitis somewhat resembling a cirrhosis,¹² a myocarditis with scar formation and a glomerular and a parenchymatous nephritis¹³ in rabbits, cats, guinea-pigs, and dogs by repeated sensitization with proteins. The frequency with which myocardial and renal lesions are found accompanying and following acute and chronic tonsillar conditions has long been known. These secondary conditions have been attributed usually to a systemic bacterial invasion with the tonsil as the atrium. This, no doubt, is, in most cases, the predominant etiological factor. It does not seem improbable, however, that some of these conditions may be due, in part at least, to toxic protein products of tonsillar source.

12. *Trans. Assn. Am. Phys.*, 1913, 28, p. 98.

13. *Jour. Exper. Med.*, 1913, 18, p. 678.